



# DEFICIENCY OF VITAMIN D IN YOUNG NORTH INDIAN FEMALES AND THE EFFECT OF CONSERVATIVE RELIGIOUS CLOTHING ON VITAMIN D STATUS

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## ABSTRACT

**Objective:** This study aims to investigate the influence of conservative religious clothing on the serum 25(OH) vitamin D levels in young North Indian healthy females and to compare the same to levels obtained in those who adopt less restrictive 'western' style apparel. **Materials and Methods:** Serum samples were obtained from 25 women who observed scrupulous veiling and 25 women who did not observe any degree of veiling. Chemiluminescence assays were used to analyze the samples. Student's t-test was utilized in the comparison. **Results:** All but one of the participants had vitamin D deficiency (25(OH) D levels <20 ng/ml). Observed mean values in both groups were very low. While the non-veiled group had a mean serum level of  $9.37 \pm 4.2$  ng/ml, the veil-practicing group had a mean level of  $8.41 \pm 3.23$  ng/ml. Although, there was a slight difference in mean levels, it was not statistically significant. On comparison of the two study groups, the t-value was found out to be 0.90266 while the p-value was 0.371212. The result was not significant at  $p < .05$ . **Conclusions:** The effect of orthodox religious clothing seems to have little, if any influence on serum vitamin D levels, especially when sun exposure and dietary patterns are equally improper in both the groups. Consumption of fortified foods, supplements and adequate sun exposure is recommended on a primary basis and takes precedence over clothing habits to augment vitamin D levels in north Indian women.

**KEYWORDS:** Vitamin D, deficiency, Student's t-test.

## INTRODUCTION:

Of late, Vitamin D has been making waves amongst medical researchers. Vitamin D is the collective name given to a series of 'prohormone' vitamers, of which D2 (ergocalciferol) and D3 (cholecalciferol) are biologically active and well-studied compounds (Norman, 2008). Although, other forms such as D1, D4 and D5 have also been isolated, D2 and D3 are of prime importance in human biology. Unless otherwise stated, vitamin D without the subscript conventionally refers to D2 and D3. Both of these compounds are secosteroids and have minimal structural differences, although potency of D3 more or less outweighs that of D2 (Cashman, 2012). D2 is artificially prepared by irradiating ergosterol obtained from yeast (Holick, 2005).

Most of the supply of this 'sunshine vitamin' is from the action of Ultraviolet light (UV-B portion) present in abundance in the sun's rays, on 7-dehydrocholesterol in the skin, resulting in thermal isomerization and the subsequent synthesis of cholecalciferol (D3) which is hydroxylated in the liver initially to calcidiol, which serves as the storage form, followed by further hydroxylation in the kidneys to yield calcitriol or 1,25-dihydroxycholecalciferol, the active form of vitamin D. 25(OH)D<sub>3</sub>-1- $\alpha$  hydroxylase is the enzyme that converts calcidiol into calcitriol in the kidneys (and any other tissues harbouring it) (Lips, 2006). A vitamin D binding protein albumin in serum, namely DBP, conveys the various 25(OH) hydroxylated vitamers to target tissues (Bikle, Adams, & Christakos, 2009). These other metabolites such as 24,25(OH)<sub>2</sub>D<sub>3</sub> and even calcidiol also exert debatable influence on bone and mineral metabolism although the existence of receptors for vitamers other than calcitriol is still not conclusive (Kumar & Malhotra, 2014).

Of the 37 or so metabolites of Vitamin D, calcitriol appears to be most significant and is transported to target organs where it binds with nuclear receptors, plasma membrane receptors or both, thus propagating its action. Also, 25(OH)D<sub>3</sub>-1- $\alpha$  hydroxylase is not only present in the kidneys but also present in atleast 10 tissues where it acts locally to produce calcitriol (Although spillover effects are not ruled out). This metabolite is designated for 'local consumption' in the tissue and is usually not involved in the larger aspect of bone-mineral metabolism (Norman, 2008).

Calcitriol acts similarly to steroid hormones, binding intracellularly to the Vitamin D receptor (VDR) which has similarities with other hormone receptors. VDR along with retinoid X receptor (RXR) forms a heterodimer and subsequently activates specific sequences, the vitamin D response elements (VDRE) resulting in transcriptional control (Bikle et al., 2009).

Vitamin D has significance for the abovementioned bone-mineral metabolism as it influences calcium and phosphate homeostasis, and possesses extra-skeletal effects as well, influencing as many as 2,000 genes. There are around 36 organs that express vitamin D receptors. Prominent among the extraskeletal effects are its anti-proliferative effects in cancer and its immunomodulatory role in various auto-immune as well as infectious diseases. The adaptive as well as innate

immune system seem to be under its influence. Optimal levels of this vitamin are essential for optimal immune function. An extension of this may be the 'soothing effect' of Vitamin D supplementation on airway hyperreactivity in asthma exacerbations. In addition, there is significant proof of its role in mitigating cardiovascular risk as well as the development of overt diabetes. Even a neuropsychiatric role has been suggested in many cases (Wacker & Holick, 2013). Synthesis of vitamin D in the kidneys is under regulation of parathyroid hormone (PTH), serum calcium and phosphate, as well as the Fibroblast Growth Factor-23 (FGF-23), all of which exert a negative feedback.

Renal production of 1,25(OH)2D is regulated by parathyroid hormone (PTH), as well as serum calcium and phosphorus concentrations. When calcium levels are too low, PTH and vitamin D act synergistically to increase blood calcium by increasing reabsorption of dietary calcium and releasing calcium and phosphorus from bone

A serum 25(OH) D<sub>3</sub> level of atleast 30–40 ng/mL imparts a 'protective' or 'prophylactic' effect on people at risk of injury to bone, carcinomas as well as a host of autoimmune diseases. Levels between 20–29.99 ng/ml indicate insufficiency while values less than 20 ng/ml indicate deficiency (Iruretagoyena, Hirigoyen, Naves, & Burgos, 2015). Other studies emphasize serum levels of atleast 80 nmol/L (32 ng/ml) to 120 nmol/L (48 ng/ml) for vitamin D to exert its full effects (Cashman, 2012). Any level below 50 nmol/L (20 ng/ml) places an immense risk of derangements in bone as well as glucose homeostasis and possible development or worsening of insulin resistance and metabolic syndrome. Although the cut-off of 30 ng/ml has been advised for bone health, a fixed lower limit for non-skeletal health is yet forthcoming (Chirita-Emandi et al., 2015; Alemzadeh, Kichler, Babar, & Calhoun, 2008).

As sunlight intensity varies with latitude, cloud cover, season and time of the day, vitamin D is thus synthesized in a fluctuating manner in the skin. Additionally, a pleomorphic skin pigmentation secondary to inter and intra-racial variations in individuals results in differential rates of UV absorption. Sunscreen application drastically reduces production of cholecalciferol. Iruretagoyena et al highlighted the lack of awareness that the prime source of Vitamin D is sunlight exposure, primarily during the summers and that only around 10% is usually obtained from the diet, mostly through animal sources and oily fish, although many foods especially in western countries are being fortified with varying amounts. Even these fortified foods may be too meagre as contributors. Cashman stated that areas above 35–37° N latitude are especially prone to shortfalls in cutaneous D<sub>3</sub> production in winter. In such areas, minimal cholecalciferol is produced from 7-dehydrocholesterol despite clothless skin exposure. In fact, absolutely nil cutaneous vitamin D production was observed in a study at 52°N latitude for the period October-April and another study at 42°N latitude for the period November-February (CPS, 2007). Calcidiol is stored in large amounts in the adipose tissue and 'withdrawals' are made from this deposit during the season of scarcity, viz. the winter. Unfortunately, obese individuals also tend to have 'deeper' and inaccessible deposits of this pro-hormone and are able to raise levels by only approx-

imately half as that raised in non-obese subjects (see Holick, 2005; Alemzadeh et al., 2008).

Studies in white subjects show that receiving one minimal erythema dose (MED), which imparts a slight pink hue to the skin, and is usually obtained at latitudes closer to the equator within 20-40 minutes, results in a rise in serum vitamin D level equivalent to that obtained by taking 10,000-20,000 IU of vitamin D2 orally (If the variation in potency or biological activity of D2 in comparison to D3 is to be ignored). Holick's study specified a daily intake of 3000-5000 IU from both diet and sun exposure combined would be sufficient for availing optimum and additional benefits of D3. Bikle et al. emphasized that overexposure to the sunlight beyond the recommended duration does not appear to increase serum vitamin D levels, rather, other inactive metabolites such as lumisterol and tachysterol accumulate. Neither does toxicity occur from chronic exposure to sunlight, although the risk of certain skin cancers definitely increases.

Alemzadeh et al stated that amount of melanin in the skin is negatively correlated with serum 25(OH) D<sub>3</sub> levels. This appears to put dark-complexioned individuals at a disadvantage vis-a-vis cutaneous D3 production potential. Were it not for the abundant sunlight and the optimum angle of the sun's rays in the equatorial regions where most of the intensely pigmented populations live, vitamin D<sub>3</sub> levels would have been grossly low in comparison to Caucasian populations. 'Asian osteomalacia' is actually a novel term given to Asian migrants to Northern European countries who suffer from a more severe hypovitaminosis in comparison to the local Caucasian population, primarily because of the higher pigmentation, but clothing habits might be contributory as well. Some studies show that the mean vitamin D levels in Arab females are generally slightly lower than those obtained in males (Al-Horani et al., 2016) This could be attributable to more conservative clothing habits, confinement indoors or more body fat percentage. Also, non-vegetarians have somewhat elevated levels compared to vegetarians of varied dietary denominations (Glerup et al., 2000; Lamberg-Allardt, Kärkkäinen, Seppänen, & Biström, 1993).

#### MATERIALS AND METHODS:

The study was conducted during the months of June, July and first half of August 2017, which had ample sunshine and/or rain interspersed with exceedingly hot and cloudless days. A total of 52 female subjects between the ages of 18-32 were included in the study. 25 healthy undergraduate and postgraduate students were taken as the 'unveiled' controls, who never utilized any traditional or conservative head/body covering such as a veil or scarf and wore western apparel with ample potential for sunlight exposure. 27 veiled subjects were taken who had adopted more orthodox apparel and had habitually worn the full body veil or 'burkha' as it is colloquially referred to. Most of the veiled subjects did not cover the face or covered it only partially. 2 veiled subjects had to be excluded from the study due to several instances of consumption of calcium and vitamin D<sub>3</sub> supplements in the preceding few months. Both the cases and controls lived mostly between the latitudes of 27° to 30° N during the greater part of the preceding year. There was some hesitation in some cases on the phlebotomy procedure but it was negated by motivation and reassurance.

#### Laboratory measurements:

Samples for measuring 25 OH cholecalciferol were drawn between 9 am to 10 am (after an overnight fast) by venepuncture under all aseptic precautions. At least 3 ml blood was obtained. Serum was separated after centrifugation and sample was analyzed within 2 hours of venepuncture and the results obtained and intimated to the patient within 3 hours of venepuncture.

#### Serum vitamin D [25(OH)D]:

It was assayed by direct competitive chemiluminescence immunoassay (CLIA). The biochemical investigation was carried out by Siemens ADVIA Centaur XP Immunoassay System fully-automated machine that is in the endocrinology laboratory of the biochemistry department in the hospital of MMIMSR of Mullana, Ambala, Haryana. ("ADVIA Centaur Vitamin D Total Assay Specifications [Package insert]," 2013)

#### Other data:

The height and weight of the individuals was also obtained and the body mass index (BMI) calculated accordingly. A value between 18.5-25 was taken as the normal BMI.

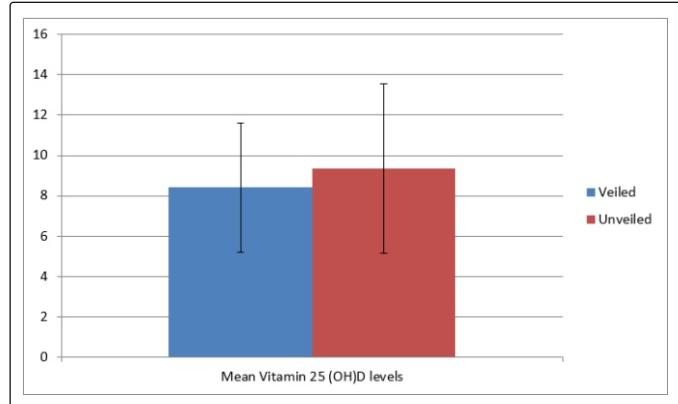
#### Statistical analysis:

Data collected was entered into Microsoft Excel worksheet and statistically analysed by using SPSS (Statistical Package for Social Sciences) version 20. Proportions were expressed as percentage & student's t-test (2-tailed) was used to compare the two groups.

#### RESULTS:

24 out of 25 unveiled subjects had vitamin D deficiency (25(OH) D levels <20 ng/ml) while only one had levels above 20 ng/ml. All of the veiled subjects had vitamin D deficiency. Thus, actually none of either the veiled or unveiled had optimum vitamin D levels (25(OH) D levels >30 ng/ml). Observed mean values in both groups were very low. The unveiled group had a mean serum level of 9.37 ± 4.2 ng/ml, while the veiled group had a mean level of 8.41 ± 3.23 ng/ml. Although, there was a slight difference in mean levels, it was not statistically significant.

On comparison of the two study groups, the t-value was found out to be 0.90266 while the p-value was 0.371212. The result was not significant at  $p < .05$ .



#### Group Statistics

	gp	N	Mean	Std. Deviation	t-value
age	unveiled	25	22.08	3.239	.359
	veiled	25	21.76	3.059	
weight	unveiled	25	56.880	13.4298	1.699
	veiled	25	51.020	10.8210	
height	unveiled	25	157.04	4.962	-1.403
	veiled	25	159.20	5.888	
BMI	unveiled	25	22.9016	4.71231	2.553
	veiled	25	19.9800	3.24654	
vitamin D	unveiled	25	9.3732	4.20317	.903
	veiled	25	8.4152	3.23921	

#### DISCUSSION:

Veiling invariably acts as a highly efficacious sunscreen. Several studies have repeatedly explained the paucity of serum D levels in scrupulously veiled women. In a study involving 217 asymptomatic Lebanese women, it was observed that veiled subjects had significantly lower serum 25(OH) D3 levels, higher body mass index (BMI) and lower dietary vitamin D intake as compared to unveiled controls. In a Turkish study, veiled women had lower vitamin D levels compared to unveiled controls. These subjects were mostly less educated, less physically active and stayed mostly indoors. However, there were no indiscreet evidences of osteomalacia or gross vitamin D insufficiency. In another somewhat conflicting study conducted in Bangladesh, not much difference was noted in veiled women vis-a-vis unveiled women. Homebound lifestyles in both the case and control groups coupled with atmospheric pollution blocking the sun's rays produced equal reductions in both the groups. Fortunately a fish based diet in both groups seemed to offset the influence of these factors and the levels were found to be remarkably adequate, if not marginally deficient. Apparently dietary compensation has a role. In a Saudi study, 80% of veiled premenopausal women in the sample group had vitamin D deficiency attributable to the customary clothing pattern. The hot weather, discouraging outdoor traversal as well as the comparatively darker skin pigmentation acted as 'compounding factors'.

In a 2014 study in Turkey, conservative Islamic dressing which had only the hands and face being uncovered was associated with lower vitamin D levels in comparison to females who adopted western clothing. There was also some association with the age at which the veil was adopted by the individual.

In India, the common perception of being a sunny 'vitamin D' friendly country is misplaced. Studies among Indian medical professionals actually revealed deficiencies in both the northern as well as the southern parts of the country. At various latitudes in the Indian subcontinent, marked deficiencies of vitamin D persist. Studies showed strikingly low levels in children in New Delhi (28.3° N), as well as adults of both sexes in Tirupathi, which is much closer to the equator at 13.4° N. A particular study conducted at 26° N revealed that almost 90% of adolescent girls and 74% of pregnant women in a rural area were deficient, with the shortfall drastically prominent in the winter months.

#### CONCLUSION:

Our study confirmed the endemic deficiency of vitamin D in young females, despite living at lower latitudes. That veiling had negligible effects on serum vitamin D levels shows that other factors are at play here. Both the groups studied or worked at the same institution and had a broadly similar level of participation in outdoor activities. That levels were far below healthy levels in both the groups points to either inadequate dietary intake of foods rich in vitamin D or avoidance of sun exposure. The increased adoption of sunscreen lotions and the aesthetically motivated avoidance of direct sun exposure to avoid tanning, influenced by beauty fads, may have the most significant contribution towards the extremely

inadequate levels observed in these young females. That none of the participants had any medical complaints or apparent symptoms of any infirmity or ailment indicate that the optimum minimum level of vitamin D in the population living at this latitude in northern India may need further study. Indian standards of vitamin D supplementation may actually entail higher doses of vitamin D supplements than that stipulated by studies conducted in western countries. Nonetheless, regular oral vitamin D supplementation or daily consumption of foods fortified in this compound may be of value in skeletal health and especially in non-skeletal health, the effects of which may not be immediately apparent but would definitely add to the quality of life of Indian women.

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